

Molecular Weight Estimation

Run the standards and samples on an SDS-PAGE gel. Process the gel with the desired stain and then destain to visualize the protein bands. Determine the R_f graphically or using Quantity One® analysis software (or equivalent).

- 1 Using a ruler, measure the migration distance from the top of the resolving gel to each standard band and to the dye front.
- 2 For each band in the standards, calculate the R_f value using the following equation:
 $R_f = \text{migration distance of the protein} / \text{migration distance of the dye front}$
- 3 Repeat this step for the unknown bands in the samples.
- 4 Use a graphing program, plot the $\log(\text{MW})$ as a function of R_f .
- 5 Generate the equation $y = mx + b$, and solve for y to determine the MW of the unknown protein.

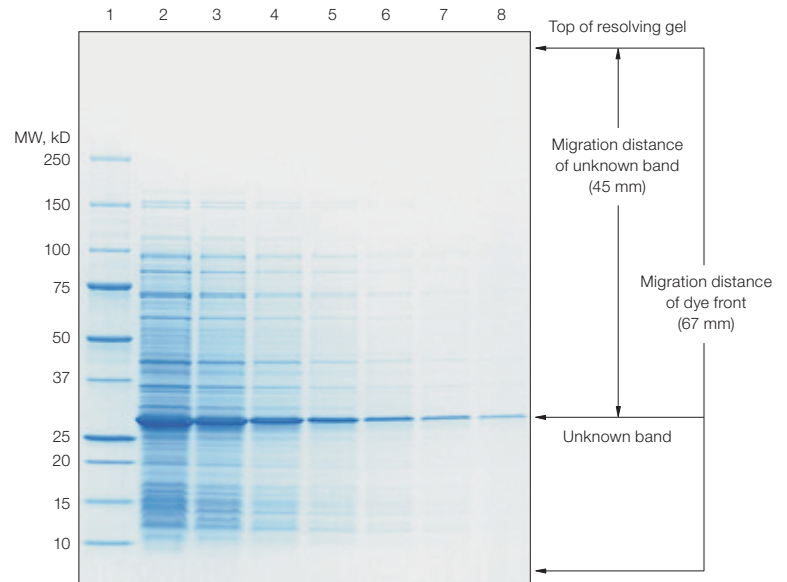


Fig. 1. Example showing MW determination of an unknown protein. Lane 1, 10 μl of Precision Plus Protein™ unstained standards; lanes 2–8, a dilution series of an *E. coli* lysate containing a hypothetical unknown protein (GFP). Proteins were separated by SDS-PAGE in a Criterion™ 4–20% Tris-HCl gel and stained with Bio-Safe™ Coomassie stain. Gel shown is the actual size.

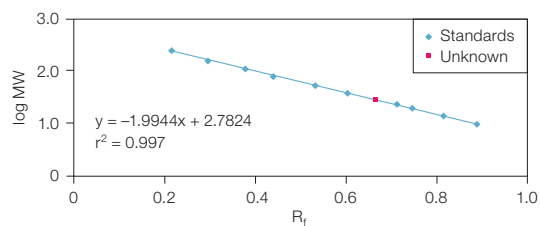


Fig. 2. Determining the MW of an unknown protein by SDS-PAGE. A standard curve of the $\log(\text{MW})$ versus R_f was generated using the Precision Plus Protein standards from Figure 1. The strong linear relationship ($r^2 > 0.99$) between the proteins' MW and migration distances demonstrates exceptional reliability in predicting MW.

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6040).



**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Web site www.bio-rad.com **USA** 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 01 877 89 01 **Belgium** 09 385 55 11 **Brazil** 55 11 5044 5699
Canada 905 364 3435 **China** 86 21 6169 8500 **Czech Republic** 420 241 430 532 **Denmark** 44 52 10 00 **Finland** 09 804 22 00
France 01 47 95 69 65 **Germany** 089 31 884 0 **Greece** 30 210 9532 220 **Hong Kong** 852 2789 3300 **Hungary** 36 1 459 6100 **India** 91 124 4029300
Israel 03 963 6050 **Italy** 39 02 216091 **Japan** 03 6361 7000 **Korea** 82 2 3473 4460 **Mexico** 52 555 488 7670 **The Netherlands** 0318 540666
New Zealand 64 9 415 2280 **Norway** 23 38 41 30 **Poland** 48 22 331 99 99 **Portugal** 351 21 472 7700 **Russia** 7 495 721 14 04
Singapore 65 6415 3188 **South Africa** 27 861 246 723 **Spain** 34 91 590 5200 **Sweden** 08 555 12700 **Switzerland** 061 717 95 55
Taiwan 886 2 2578 7189 **Thailand** 800 88 22 88 **United Kingdom** 020 8328 2000
